Evaluation of a Killed Phase I *Coxiella burnetii* Vaccine in Cynomolgus Monkeys *(Macaca fascicularis)*[1,2,3]

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Summary | The protective efficacy of a killed, purified, phase I *Coxiella burnetii* vaccine was tested in cynomolgus monkeys. Monkeys vaccinated once with 30 μg of the antigen were challenged 6 or 12 months later with virulent phase I rickettsiae administered in small-particle aerosols. The vaccine provided only partial protection, since some of the challenged monkeys developed clinical signs of illness. However, the vaccinated animals did not develop pneumonia as determined by radiographic evaluation nor any hematologic or chemical changes except for an increase in fibrinogen. Although rickettsiae were isolated from peripheral blood in vaccinated monkeys, the rickettsemia persisted for only 1-2 days; whereas, organisms were recovered from unvaccinated animals for 6-7 days. All vaccinated animals had circulating microagglutinating antibodies to phase I and phase II antigens 6 and 12 months after vaccination.

Key Words | *Coxiella* — Vaccine — *Macaca*

A host-controlled phase variation of *Coxiella burnetii*, that is, naturally occurring phase I and phase II variants which appear following a variable number of passages in embryonated chicken eggs, has been reported previously (1). Q fever vaccines prepared from strains of killed *C burnetii* predominantly in phase II have been shown to be effective in eliciting a humoral antibody response and protection of humans and guinea pigs (2,3). However, a serious problem with these phase II vaccines has been the high incidence of local and systemic reactions (3,4).

Since *C burnetii* occurs in nature as phase I, it would seem appropriate to use phase I-derived vaccines. In support of this hypothesis, it was previously shown (5) that vaccines made from strains of purified phase I *C burnetii* had protective potencies 100-300 times greater than comparable phase II vaccines in guinea pigs challenged with phase I organisms.

A significant number of human volunteers vaccinated with doses varying from 1 to 150 μg of a killed phase I *C burnetii* vaccine (either in a single dose or three doses) developed mild erythema and induration, but no serious local or systemic reactions (6,7). Subjects immunized with a single dose of 30 μg of phase I vaccine developed humoral antibody; approximately 90% of them were protected against an aerosol challenge with phase I rickettsiae 5-10 months later (7).

Although guinea pigs are usually recognized as the animal model of choice for Q fever infection, the evaluation of vaccines for ultimate use in humans is frequently performed in nonhuman primates.

Materials and Methods

Rickettsial strain: The third egg passage of the phase I Henzerling strain of *C burnetii* was grown in chicken embryo cells for challenge studies as previously described (8).

Vaccine: A purified, particulate, formalin-inactivated, third egg-passaged, Henzerling strain phase I *C burnetii* was used in this study (9).

Monkeys: Twenty, young adult, feral cynomolgus monkeys *(Macaca fascicularis)* of both sexes, weighing 2.0-3.5 kg were housed in individual cages and provided commercial ration and water *ad libitum*. None of these animals, which had been in captivity for 1 year, had prior history of exposure to *C burnetii* or detectable antibody to phase I or II *C burnetii*.

Experimental design: Sixteen monkeys were tested with an intradermal injection of 0.1 ml (0.02 μg) of the vaccine into the left palpebrum to identify hypersensitive animals and possible pre-existing immunity; none of the animals developed a delayed type hypersensitivity. Monkeys were vaccinated subcutaneously 1 week later with 0.5 ml of vaccine (30 μg). Thirty days later, monkeys were retested with 0.1 ml of antigen given intradermally into the right palpebrum. An additional four monkeys were used as unvaccinated controls.

Aerosol dissemination and sampling: Six and 12 months following vaccination, eight vaccinated and two unvaccinated control monkeys were challenged with 10^5

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[3]The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.
[4]NDBR 105, lot 4, Salk Institute (formerly National Drug), Swiftwater, PA
[5]Wayne Monkey Chow®, Wayne Feed Supply Co, Gaithersburg, MD

mouse median intraperitoneal infectious doses ($MIPID_{50}$) of phase I Henzerling strain of *C burnetii* administered in small-particle aerosols as previously described (**10**).

Clinical observations: Hematology, radiographic evaluation, and serum chemistry determinations on monkeys were performed by previously described methods (**11**). To assess rickettsemia, heparinized monkey blood was diluted in heart infusion broth; 1.0 ml of each diluted sample was inoculated intraperitoneally into four white Swiss mice [Tac:(SW)fBR].* Twenty-one days later, the mice were bled; seroconversion was measured by indirect immunofluorescence (**12**). Serum samples from monkeys were collected prevaccination, prechallenge, 1, 2, and 3 weeks, and 6 months after challenge for measurement of humoral antibodies to phase I and II *C burnetii* by the microagglutination technique (**13**).

Necropsies and histopathologic examinations: Four unvaccinated control monkeys were necropsied 21 days after challenge. Tissues were fixed in 10% neutral phosphate-buffered formalin, imbedded in paraffin, sectioned, and stained with hematoxylin and eosin. No vaccinated monkeys were necropsied, since this was part of a long-term study on vaccine effects.

Statistical analysis: Analysis of variance for repeated measurements was used to detect differences significantly greater than any variability of individual baseline values. Significance of repeated measurements was based on the least significant difference procedure, with the lowest level and significance set at $p = 0.05$.

Results

Clinical responses: All unvaccinated control animals developed anorexia, depression, and fever 5-8 days following challenge. None of the eight vaccinated monkeys challenged at 6 months became clinically ill. Two of the eight animals challenged at 12 months developed anorexia and depression. Six of the vaccinated animals developed only a mild, transient fever between 4 and 6 days following challenge. There was no significant difference in the incubation time or duration of fever between control and vaccinated monkeys. All the unvaccinated control and six of the vaccinated animals showed an increase in respiratory rate (60-80 breaths/minute) 4-6 days after challenge ($p = 0.05$), compared to the normal rate of 25-35 breaths/minute. Duration of tachypnea was 1-2 days in the vaccinated monkeys, compared to 5-7 days for unvaccinated controls. Monkeys vaccinated 6 and 12 months previously showed no significant changes in hematology and serum chemistry values. Control animals developed absolute neutrophilia and lymphopenia, increased alkaline phosphatase, serum glutamic oxalacetic transaminase, and total bilirubin. These results are in agreement with our previous study (**11**). Plasma fibrinogen levels rose concurrently with elevated temperature in both vaccinated and control monkeys. Thoracic radiographs of all vaccinated animals showed no discernible changes, while control monkeys developed signs of severe interstitial pneumonia.

*Taconic Farms, Germantown, NY

Unvaccinated control monkeys developed rickettsemia 4 days after challenge; organisms persisted in peripheral blood for 11 days. In contrast, rickettsiae were observed only between days 4 and 6 in vaccinated animals (Figure 1). Rickettsiae recovered from control animals were as many as 100 times more numerous than from vaccinated monkeys.

Reactogenic and hypersensitivity reaction: None of the monkeys developed local or systemic reactions to the 30-μg vaccination dose. No delayed type hypersensitivity was observed 30 days after vaccination. Monkeys that had been vaccinated and then challenged were again skin tested on the chest with varying doses 3 months post-challenge. A skin test dose of 0.02 μg elicited no delayed type hypersensitivity. Slight edema and reddening occurred with a 2.0-μg dose; pronounced reactions (> 10 mm induration) occurred at 48 hours following 30 μg, (5 of 8 monkeys). Even at this high level, induration was moderate, and no abscesses were observed.

Pathology: Unvaccinated control monkeys were necropsied at 21 days post-exposure. Microscopic examination of tissues revealed moderate to severe interstitial pneumonia and mild to moderate multifocal granulomatous hepatitis in all four monkeys.

Antibody response: Antibody titers following vaccination and challenge are summarized in Figure 2. Microagglutinating antibodies to phase I and II antigens were detected 1, 2, and 4 months after vaccination, and at 6 months, just prior to challenge. Following challenge, a rapid increase in titer to both phase I and II antigens at 1 week was observed, and titers persisted at high levels for 6 weeks. Similarly, antibodies to phase I and II antigens also were present after vaccination and prior to challenge in animals challenged at 12 months. Following challenge, there was an increase in phase II antibodies 1 week later, but phase I antibodies were not detected until week 3. In contrast to the group challenged at 6 months, antibody

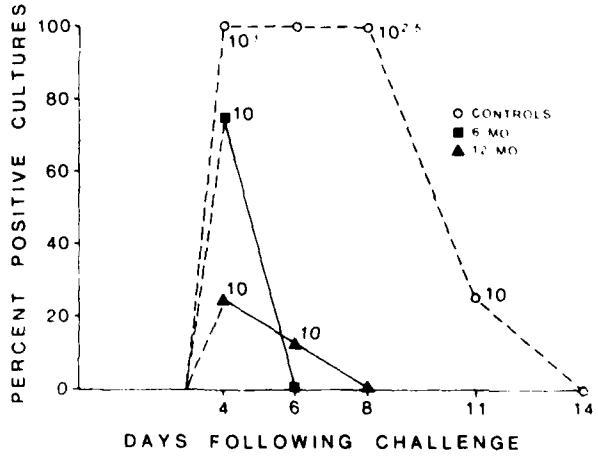

Figure 1
Isolation of rickettsiae from peripheral blood of vaccinated and unvaccinated monkeys following challenge (mean number of rickettsiae per milliliter of blood).

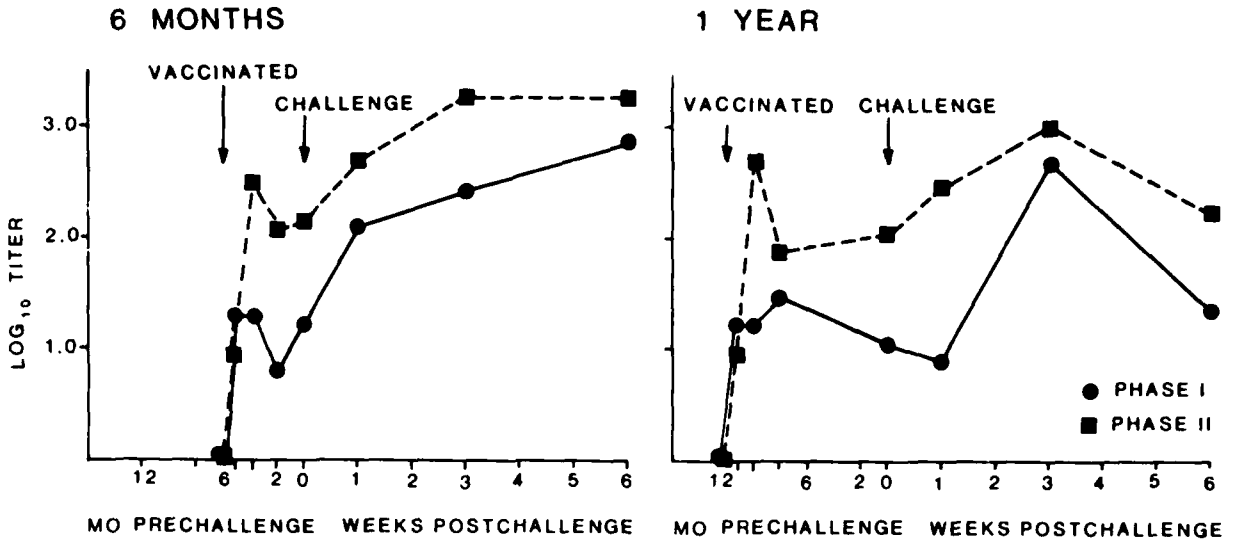

Figure 2
Mean microagglutination titers of monkeys 6 month (left) and 1 year (right) after vaccination.

levels began to decline by week 6 in those monkeys challenged at 12 months.

Discussion

The phase I *C burnetii* vaccine suppressed infection but provided only partial protection, since some of the vaccinated monkeys developed clinical signs of illness. Vaccinated monkeys did not become severely depressed and anorectic and had less severe tachypnea than unvaccinated monkeys. There were no changes in thoracic radiographs and hematologic and serum chemistry values in vaccinated monkeys.

This vaccine significantly reduced the duration of rickettsemia and concentration of organisms. Similarly, others (**14**) have observed that vaccination of cows with a phase I vaccine greatly reduced the shedding of rickettsiae from milk, colostrum, and the placenta.

The most pronounced histologic lesions in unvaccinated control monkeys were moderate to severe interstitial pneumonia and multifocal hepatitis. These changes were compatible with those previously reported in monkey (**11**) and man (**15**). Although none of these vaccinated monkeys were necropsied, radiographic examination indicated no pulmonary changes following challenge at 6 and 12 months. Monkeys vaccinated 6 and 12 months previously had relatively high antibody titers to phase I and II *C burnetii* prior to challenge, but most developed mild febrile responses and rickettsemia. There was a rapid anamnestic response in vaccinated animals following challenge.

Although there is no direct evidence that a positive skin test measures protective immunity to *C burnetii*, delayed type hypersensitivity has been used as a reliable indicator of prior natural infection or previous vaccination (**6**). Most men injected one to three times with a killed phase I vaccine developed circulating antibody and a positive skin test (**6**). In the present study, none of the monkeys tested 30 days after vaccination developed delayed type hypersensitivity with a skin test dose of 0.02 μg. Monkeys that were vaccinated, challenged at 6 months, and skin tested again at 3 months had only minor reactions with a test dose of 2.0 μg. However, they had a moderate reaction, with erythema and induration, using a test dose of 30 μg. The hyporesponsiveness observed in our monkeys may be similar to that observed by others (**16**) unable to detect a delayed reaction in several species of nonhuman primates to *Monilia*, *Trichophyton*, streptokinase-streptodornase, and mumps antigens.

One of the most serious problems associated with phase II vaccines is the high incidence of local and systemic reactions, including erythema, induration, fever, headache, nausea, and sterile abscesses (**4**). It has been estimated that as many as 26.5% of vaccinees had local reactions after receiving the recommended regimen of three doses given 1 week apart, and 0.8% had abscesses at the site of injection (**4**). The incidence of abscesses was usually higher among individuals who had been previously vaccinated or exposed to the virulent agent (**3**). Previous human studies with phase I antigens demonstrated minimal, but acceptable reactions following one to three doses (**6–8**). In contrast to the serious problem of local or systemic reactions associated with phase II vaccines, we did not observe significant reactions to the administration of the phase I vaccine in our test animals. This lack of reactogenicity may be due to the fact that the phase I vaccine used is a more pure antigen than the previously available phase II vaccines. It may also be a product of the relative hyporesponsiveness in this species of nonhuman primates.

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